

Claims

1. A method of modifying cell proliferation in a plant which comprises modulating the expression of a gene whose expression or transcription product is capable of directly or indirectly modulating cell proliferation in the plant or plant propagating material, whereby cell proliferation, within the integuments and/or seed coats of the plant, is modified.
2. A method of modifying cell proliferation in a plant which comprises the step of transforming a plant, or plant propagating material, with a nucleic acid molecule comprising at least one regulatory sequence capable of directing expression within the integuments and/or seed coat of at least one nucleic acid sequence whose expression or transcription product is capable of directly or indirectly modulating cell proliferation, whereby, on expression of that sequence, cell proliferation is modified.
3. A method according to claim 1 or 2 in which the overall size of the integuments/seed coat is modified.
4. A method according to claim 1, 2 or 3 in which the function of a gene or gene product that promotes cell division is enhanced or the function of a gene or gene product that represses cell division is inhibited.
5. A method according to claim 4 in which cell division in the integuments/seed coat is increased resulting in a larger seed of the plant compared to wild type.
6. A method according to claim 5 in which the seed is at least 15% larger than wild type.
7. A method according to claim 6 in which the seed is at least 25% larger than wild type.

8. A method according to claim 7 in which the seed is at least 35% larger than wild type.
9. A method according to claim 8 in which the seed is at least 45% larger than wild type.
10. A method according to any one of claims 5 to 9 in which the seed is at least 5% heavier than wild type
11. A method according to claim 10 in which the seed is at least 10% heavier than wild type.
12. A method according to claim 11 in which the seed is at least 20% heavier than wild type.
13. A method according to claim 12 in which the seed is at least 30% heavier than wild type.
14. A method according to claim 13 in which the seed is at least 40% heavier than wild type.
15. A method according to claim 14 in which the seed is at least 50% heavier than wild type
16. A method according to claim 15 in which the seed is at least 75% heavier than wild type
17. A method according to claim 16 in which the seed is at least 100% heavier than wild type
18. A method according to claim 17 in which the seed is at least 150% heavier than wild type
19. A method according to claim 18 in which the seed is at least 200% heavier than wild type
20. A method according to any preceding claim in which the diameter of the stem of the plant is at least 10% greater than wild type.

21. A method according to any preceding claim in which the diameter of the stem of the plant is at least 20% greater than wild type.
22. A method according to any preceding claim in which the number of cells in the integuments/seed coat is increased compared to wild type.
23. A method according to claim 17 in which the number of cells in the integuments/seed coat is increased by at least 30% compared to wild type.
24. A method according to claim 18 in which the number of cells in the integuments/seed coat of the plant is increased by at least 50% compared to wild type.
25. A method according to any preceding claim in which the sepal length of the plant is sufficiently greater than wild type to inhibit flower opening.
26. A method according to claim 25 in which the sepal length is at least 20% greater than wild type.
27. A method according to claim 26 in which the sepal length is at least 50% greater than wild type.
28. A method according to any preceding claim in which the function of a gene or gene product that promotes cell division is inhibited or the function of a gene product that represses cell division is enhanced.
29. A method according to Claim 28 in which cell division in the integuments/seed coat is decreased resulting in a smaller seed compared to wild type.
30. A method according to claim 29 in which the seed is at least 15% smaller than wild type.
31. A method according to any one of claims 28-29 in which the number of cells in the integuments/seed coat is decreased compared to wild type.
32. A method according to claim 31 in which the number of cells in the integuments/seed coat is decreased by at least 30% compared to wild type.

33. A method according to claim 32 in which the number of cells in the integuments/seed coat is decreased by at least 50% compared to wild type.
34. A method according to any preceding claim in which growth or development or function of any part of the plant other than the seed is not substantially affected.
35. A method according to any one of claims 2 to 34 in which the regulatory sequence includes a promoter.
36. A method according to claim 35 in which the promoter is a constitutive promoter.
37. A method according to claim 36 in which the promoter directs gene expression in most or all cells of the plant.
38. A method according to claim 36 or 37 in which the promoter is the *35S* promoter.
39. A method according to claim 35 or 36 in which the promoter is specific, directing expression exclusively or primarily in one organ, tissue, or cell type of the plant.
40. A method according to claim 39 in which the promoter directs expression exclusively or primarily in the integuments or seed coat.
41. A method according to claim 40 in which the promoter is expressed in the pre-fertilization integuments.
42. A method according to claim 41 in which the promoter is the promoter of the *INO* or *BEL1* gene.
43. A method according to claim 42 in which the promoter is expressed in the seed coat after fertilization.
44. A method according to claim 43 in which the promoter is the promoter of the *BAN*, *TT1*, *TT2*, *TT8*, *TT12*, or *TT16* gene.

45. A method according to any one of claims 2 to 44 in which the nucleic acid sequence includes or is derived from a gene involved in hormone response, biosynthesis, translocation, or other aspects of hormone action.
46. A method according to claim 45 in which the gene is *MNT*, *IPT1*, or *ARGOS* or their orthologues.
47. A method according to any one of claims 2 to 46 in which the nucleic acid sequence includes or is derived from a core cell cycle gene.
48. A method according to claim 47 in which the core cell cycle gene is *CYCD3;1* or *CYCB1;1* or their orthologues.
49. A method according to any one of claims 2 to 48 in which the nucleic acid is a transcription factor involved in regulation of the extent or rate of cell proliferation.
50. A method according to claim 49 in which the transcription factor is *ANT* or its orthologue.
51. A method according to any one of claims 3 to 50 in which the function of a gene that modulates cell proliferation is enhanced.
52. A method according to claim 51 in which transcription of the gene is activated.
53. A method according to claim 52 in which activation of transcription results in increased levels of mRNA and/or protein encoded by the gene.
54. A method according to any claim 53 in which levels of mRNA and/or protein encoded by the gene are increased by 20% or more.
55. A method according to any preceding claim in which a plant promoter is operably linked to a coding region of the gene in the sense orientation.

56. A method according to any one of claims 3 to 55 in which the function of the gene is modulated by operably linking a plant promoter to a nucleic acid fragment from the gene to form a recombinant nucleic acid molecule such that an antisense strand of RNA will be transcribed.

57. A method according to claim 56, in which the function of a gene is modulated by introducing nucleic acid segments of the gene into an appropriate vector such that double-stranded RNA is transcribed where directed by an operably linked plant promoter.

58. A method according to claim 56 or 57 in which decreased levels of mRNA and/or protein encoded by endogenous copies of the gene are produced.

59. A method according to claim 58 in which levels of mRNA and/or protein encoded by endogenous copies of the gene are decreased by 20%, 50% or 75% or more.

60. A method according to any one of claims 56 to 59 in which the levels of mRNA and/or protein encoded by homologues of the gene are reduced.

61. A method according to claim 60 in which levels of mRNA and or protein encoded by homologues of the gene are reduced by 20%, 50% or 75% or more.

62. A method according to any one of claim 2 to 61 in which the function of the gene is modulated by operably linking a plant promoter to a 'dominant negative' allele of the gene, which interferes with the function of the gene product.

63. A method according to any one of claims 2 to 62 in which the nucleic acid sequence comprises or is derived either from wild-type MNT or a mutant form of mnt or its orthologues.

64. A method according to any preceding claim in which the plant is further modified to maintain desirable characteristics in other parts of the plant which may have been lost or modified after the modulation or transformation step.
65. A method according to claim 64 in which the desirable characteristic is fertility.
66. A method according to claim 65 in which wild type MNT function (or function of an MNT orthologue) is restored to petals and stamens of a transformed plant or an *mnt* mutant plant such that seeds have a modified phenotype but fertility is not impaired.
67. A method according to claim 66 in which the promoter of a gene that directs expression in petals and stamens but not carpels is operably linked to the wild-type *MNT* gene or an *MNT* orthologue.
68. A method according to claim 67 in which the promoter is the promoter of the AP3 gene.
69. A method according to any preceding claim in which the plant is monocotyledonous.
70. A method according to claim 69 in which the plant is a crop plant.
71. A method according to claim 70 in which the plant is *Triticum spp* (wheat), *Oryza sativa* (rice), *Zea mays* (maize), *Hordeum spp.* (barley), *Secale cereale* (rye), *Sorghum bicolor* (sorghum), or *Pennisetum glaucum* (pearl millet).
72. A method according to any one of claims 1 to 68 in which the plant is dicotyledonous.
73. A method according to claim 72 in which the plant is *Brassica napus* (oil seed rape, canola) or any other *Brassica* species used to produce oilseeds (e.g. *Brassica carinata*), *Glycine max* (soybean), *Arachis hypogaea* (peanut), *Helianthus annuus* (sunflower), *Phaseolus vulgaris* (e.g. kidney bean, white bean, black bean), *Vicia faba* (broad bean),

Pisum sativum (green pea), *Cicer arietinum* (chick pea), *Lens culinaris* (lentil), or *Linum usitatissimum* (flax, linseed).

74. A method according to any preceding claim in which the plant is subsequently bred to be homozygous for the modulated gene.

75. A method according to any one of claims 2 to 73 in which plant is bred to be heterozygous for the modulated gene.

76. A plant which comprises, within the integuments and/or seed coats of the plant, a modulated gene whose expression or transcription product is capable of directly or indirectly modulating cell proliferation in the plant or plant propagating material, whereby cell proliferation is modified

77. A plant which includes a nucleic acid molecule comprising at least one regulatory sequence capable of directing expression within the integuments and/or seed coat of at least one nucleic acid sequence whose expression or transcription product is capable of directly or indirectly modulating cell proliferation, whereby, on expression of that sequence, cell proliferation is modified.

78. A plant according to claim 76 or 77 in which the overall size of the integuments/seed coat in the plant is modified.

79. A plant according to claim 76, 77 or 78 in which the function of a gene or gene product that promotes cell division is enhanced or the function of a gene or gene product that represses cell division is inhibited.

80. A plant according to claim 79 in which cell division in the integuments/seed coat is increased resulting in a larger seed compared to wild type.

81. A plant according to claim 80 in which the seed is at least 15% larger than wild type.

82. A plant according to claim 81 in which the seed is at least 25% larger than wild type.

83. A plant according to claim 82 in which the seed is at least 35% larger than wild type.
84. A plant according to claim 83 in which the seed is at least 45% larger than wild type.
85. A plant according to any one of claims 80 to 84 in which the seed is at least 5% heavier than wild type
86. A plant according to claim 85 in which the seed is at least 10% heavier than wild type.
87. A plant according to claim 86 in which the seed is at least 20% heavier than wild type.
88. A plant according to claim 87 in which the seed is at least 30% heavier than wild type.
89. A plant according to claim 88 in which the seed is at least 40% heavier than wild type.
90. A plant according to claim 89 in which the seed is at least 50% heavier than wild type.
91. A plant according to claim 90 in which the seed is at least 75% heavier than wild type.
92. A plant according to claim 91 in which the seed is at least 100% heavier than wild type.
93. A plant according to claim 92 in which the seed is at least 150% heavier than wild type.
94. A plant according to claim 93 in which the seed is at least 200% heavier than wild type.
95. A plant according to any one of claims 76 to 94 in which the diameter of the stem of the modified or transformed plant is at least 10% greater than wild type.

96. A plant according to claim 95 in which the diameter of the stem of the plant is at least 20% greater than wild type.
97. A plant according to any one of claims 76 to 96 in which the sepal length of the plant is sufficiently greater than wild type to inhibit flower opening.
98. A plant according to claim 97 in which the sepal length is at least 20% greater than wild type.
99. A method according to claim 98 in which the sepal length is at least 50% greater than wild type.
100. A plant according to any one of claims 76 to 99 in which the number of cells in the integuments/seed coat of the plant is increased compared to wild type.
101. A plant according to claim 100 in which the number of cells in the integuments/seed coat of the plant is increased by at least 30% compared to wild type.
102. A plant according to claim 101 in which the number of cells in the integuments/seed coat of the plant is increased by at least 50% compared to wild type.
103. A plant according to any one of claims 76 to 103 in which the function of a gene or gene product that promotes cell division is inhibited or the function of a gene product that represses cell division is enhanced.
104. A plant according to Claim 103 in which cell division in the integuments/seed coat is decreased resulting in a smaller seed compared to wild type.
105. A plant according to claim 104 in which the seed is at least 5% smaller than wild type.
106. A plant according to claim 104 in which the seed is at least 10% smaller than wild type.
107. A plant according to claim 104 in which the seed is at least 15% smaller than wild type.

108. A plant according to claim 105 in which the seed is at least 25% smaller than wild type.
109. A plant according to claim 104 in which the seed is at least 5% lighter than wild type.
110. A plant according to claim 104 in which the seed is at least 10% lighter than wild type.
111. A plant according to claim 104 in which the seed is at least 15% lighter than wild type.
112. A plant according to claim 105 in which the seed is at least 25% lighter than wild type.
113. A plant according to any one of claims 76 to 112 in which the number of cells in the integuments/seed coat is decreased compared to wild type.
114. A plant according to claim 113 in which the number of cells in the integuments/seed coat is decreased by at least 30% compared to wild type.
115. A plant according to any one of claims 76 to 114 in which growth or development of any part of the plant other than the seed is not substantially affected.
116. A plant according to any one of claims 77 to 115 in which the regulatory sequence includes a promoter.
117. A plant according to claim 116 in which the promoter is a constitutive promoter.
118. A plant according to claim 117 in which the promoter directs gene expression in most or all cells of the plant.
119. A plant according to claim 117 or 118 in which the promoter is the 35S promoter.
120. A plant according to claim 116 in which the promoter is specific, directing expression exclusively or primarily in one organ, tissue, or cell type of the plant.

121. A plant according to claim 120 in which the promoter directs expression exclusively or primarily in the integuments or seed coat.

122. A plant according to claim 121 in which the promoter is expressed in the pre-fertilization integuments.

123. A plant according to claim 122 in which the promoter is the promoter of the *INO* or *BEL1* gene.

124. A plant according to any one of claims 120 to 123 in which the promoter is expressed in the seed coat after fertilization.

125. A plant according to claim 124 in which the promoter is the promoter of the *BAN*, *TT1*, *TT2*, *TT8*, *TT12*, or *TT16* gene.

126. A plant according to any one of claims 77 to 125 in which the nucleic acid sequence includes or is derived from a gene involved in hormone response, biosynthesis, translocation, or other aspects of hormone action.

127. A plant according to claim 126 in which the gene is *MNT*, *IPT1*, or *ARGOS* or their orthologues.

128. A plant according to any one of claims 77 to 127 in which the nucleic acid sequence includes or is derived from a core cell cycle gene.

129. A plant according to claim 128 in which the core cell cycle gene is *CYCD3;1* or *CYCB1;1* or their orthologues.

130. A plant according to any one of claims 77 to 129 in which the nucleic acid encodes a transcription factor involved in regulation of the extent or rate of cell proliferation.

131. A plant according to claim 130 in which the transcription factor is *ANT* or its orthologue.

132. A plant according to any one of claims 76 to 131 in which the function of a gene that modulates cell proliferation is enhanced.

133. A plant according to claim 132 in which transcription of the gene is activated

134. A plant according to claim 133 in which activation of transcription results in increased levels of mRNA and/or protein encoded by the gene.

135. A plant according to claim 134 in which levels of mRNA and/or protein encoded by the gene are increased by at least 20%.

136. A plant according to any one of claims 76 to 135 in which a plant promoter is operably linked to a coding region of the gene in the sense orientation.

137. A plant according to any one of claims 76 to 136 in which the function of the gene is modulated by introducing a nucleic acid segments of the gene into an appropriate vector such that double-stranded RNA is transcribed where directed by an operably linked plant promoter.

138. A plant according to claim 137, in which the function of the gene is modulated by operably linking a plant promoter to a nucleic acid segment from the gene, and introducing the resulting recombinant nucleic acid into an appropriate vector such that double-stranded RNA is transcribed.

139. A plant according to claim 137 or 138 in which decreased levels of mRNA and/or protein encoded by endogenous copies of the gene are produced.

140. A plant according to claim 139 in which levels of mRNA and/or protein encoded by endogenous copies of the gene are reduced by at least 50%.

141. A plant according to any one of claims 137 to 138 in which the levels of mRNA and protein encoded by homologues of the gene are reduced.

142. A plant according to claim 142 in which the levels of mRNA and protein encoded by homologues of the gene are reduced by 50% or more.

143. A plant according to any one of claims 76 to 142 in which the function of the gene is modulated by operably linking a plant promoter to a 'dominant negative' allele of the gene, which interferes with the function of the gene product.

144. A plant according to any one of claims 76 to 143 in which the modulated gene or nucleic acid sequence comprises or is derived either from wild-type *MNT* or a mutant form of *mnt* or its orthologues.

145. A plant according to any one of claims 76 to 144 in which the plant is further modified to maintain desirable characteristics in other parts of the plant which may have been lost or modified.

146. A plant according to claim 145 in which the desirable characteristic is fertility.

147. A plant according to claim 146 in which wild type *MNT* function (or the function of an *MNT* orthologue) is restored to petals and stamens of the plant or an *mnt* mutant plant such that seeds have a modified phenotype but so that fertility is not impaired.

148. A plant according to claim 147 in which the promoter of a gene that directs expression in petals and stamens but not carpels is operably linked to a wild type *MNT* gene or an *MNT* orthologue.

149. A plant according to claim 148 in which the promoter is the promoter of the AP3 gene.

150. A plant according to any one of claims 76 to 149 in which the plant is monocotyledonous.

151. A plant according to claim 150 in which the plant is a crop plant.

152. A plant according to claim 151 in which the plant is *Triticum spp* (wheat), *Oryza sativa* (rice), *Zea mays* (maize), *Hordeum spp.* (barley), *Secale cereale* (rye), *Sorghum bicolor* (sorghum), or *Pennisetum glaucum* (pearl millet).

153. A plant according to any one of claims 76 to 149 in which the plant is dicotyledonous.

154. A plant according to claim 153 in which the plant is *Brassica napus* (oil seed rape, canola), *Glycine max* (soybean), *Arachis hypogaea* (peanut), *Helianthus annuus* (sunflower), *Phaseolus vulgaris* (e.g. kidney bean, white bean, black bean), *Vicia faba* (broad bean), *Pisum sativum* (green pea), *Cicer arietinum* (chick pea), *Lens culinaris* (lentil), or *Linum usitatissimum* (flax, linseed).

155. A plant according to any one of claims 77 to 154 in which the plant has been bred to be homozygous for the modified gene.

156. A plant according to any one of claims 77 to 154 which is heterozygous for the modified gene.

157. A method for modifying cell proliferation in a plant which comprises the step of modulating the response of the plant to an auxin in which cell proliferation is modified to produce larger or smaller seeds than wild-type.

158. A method according to claim 157 in which the response to an auxin is modulated by altering the expression of an auxin response factor.

159. A method according to claim 158 in which the auxin response factor inhibits cell division.

160. A method according to claim 159 in which the auxin response factor inhibits cell division in the integuments/seed coat.

161. A method according to claim 160 in which the auxin response factor is ARF2.

162. A method according to any one of claims 157 to 161 in which the function or expression of the auxin response factor is inhibited.

163. A method according to claim 162 in which the function or expression of the auxiliary response factor is specifically inhibited in the integument/seed coat of the plant.

164. A method according to any one of claims 157 to 163 in which a gene encoding the auxin response factor is modified so as to affect expression of the factor.

165. A method according to claim 164 in which the gene is *MNT* or an orthologue thereof.